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Peptide-based Sensing Utilizing Gold Nanoparticles for the Development of a Protease Biosensor



Biosensors Research Group
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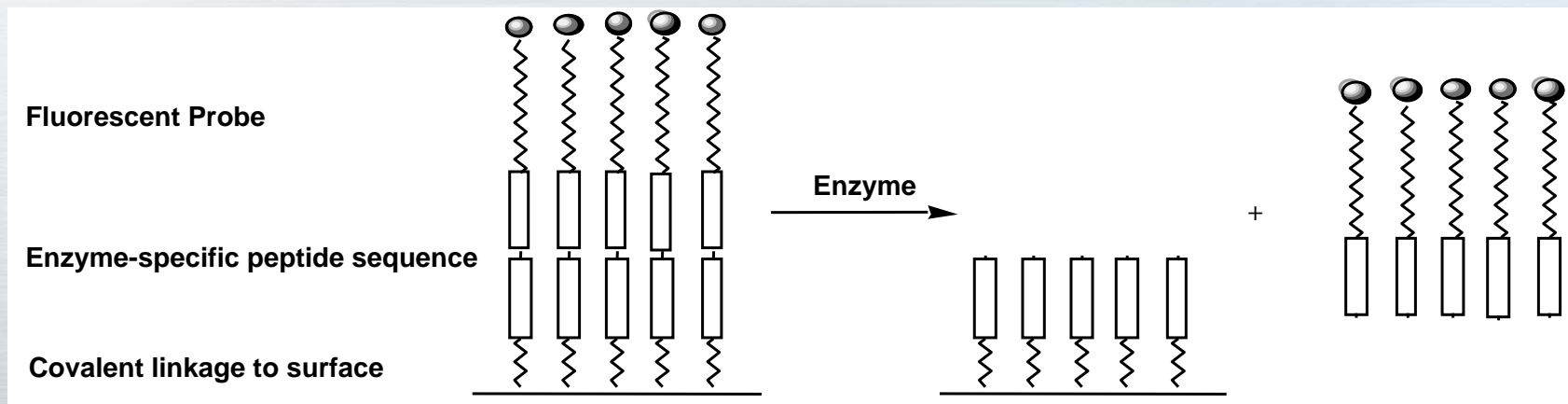
Outline

- Introduction
- Methodology
- Results
- Conclusion
- Future



Introduction

- Development of a fluorescent peptide-based biosensor
- Detection of medical relevant analytes:
 - Human thrombin/trypsin



Methodology

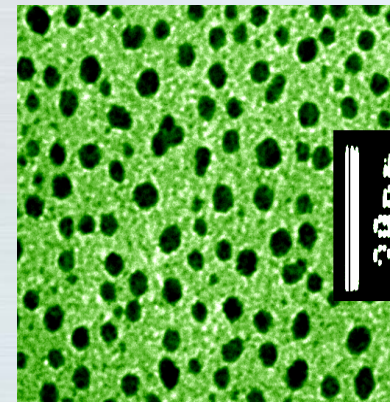


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Gold Nanoparticles

- Au Nanoparticles can be utilized as:
 - Fluorescent enhancers
 - Fluorescent quenchers
- Depends on:
 - Size
 - Shape
 - Distance
 - Orientation of dipoles



2nm-20nm
Plasmon absorption



Peptide Sequences

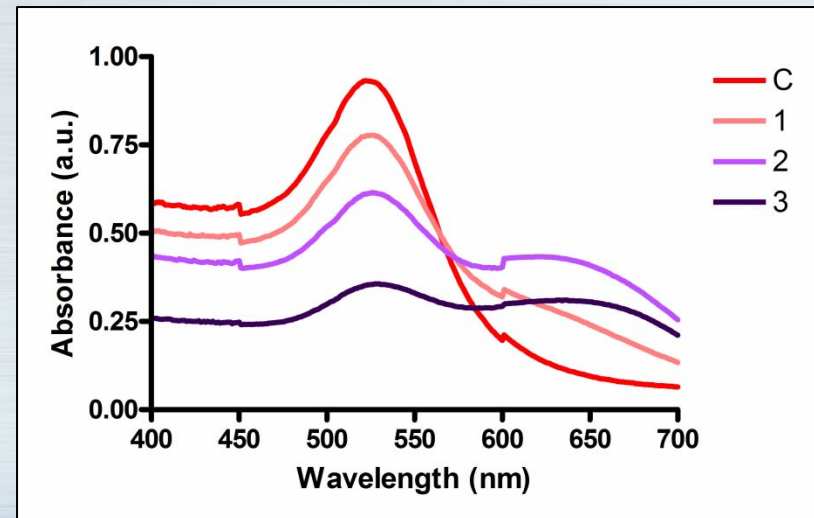
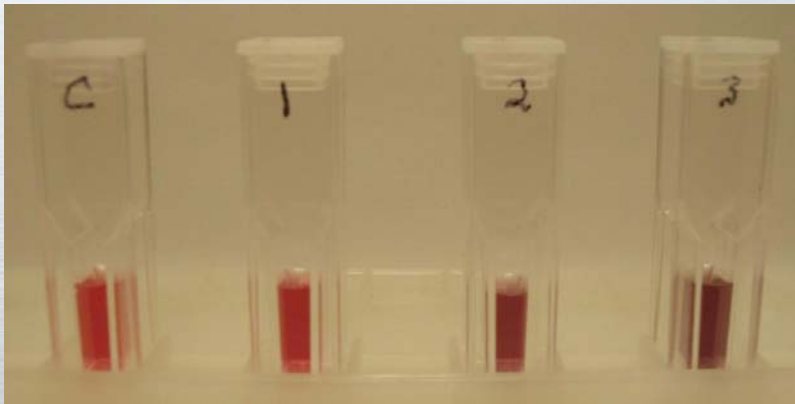
Name	Sequence
CF6	H ₂ N-Cys-dPhe- Pro-Arg-Gly -Lys(Ahx-Fluorescein)-OH
51-4	H ₂ N-Cys-Gly-Val-Pro-Gly-Val-Lys(Ahx-Fluorescein)-OH

Peptide was cleaved at enzyme sensitive region
Proline-Arginine-Glycine

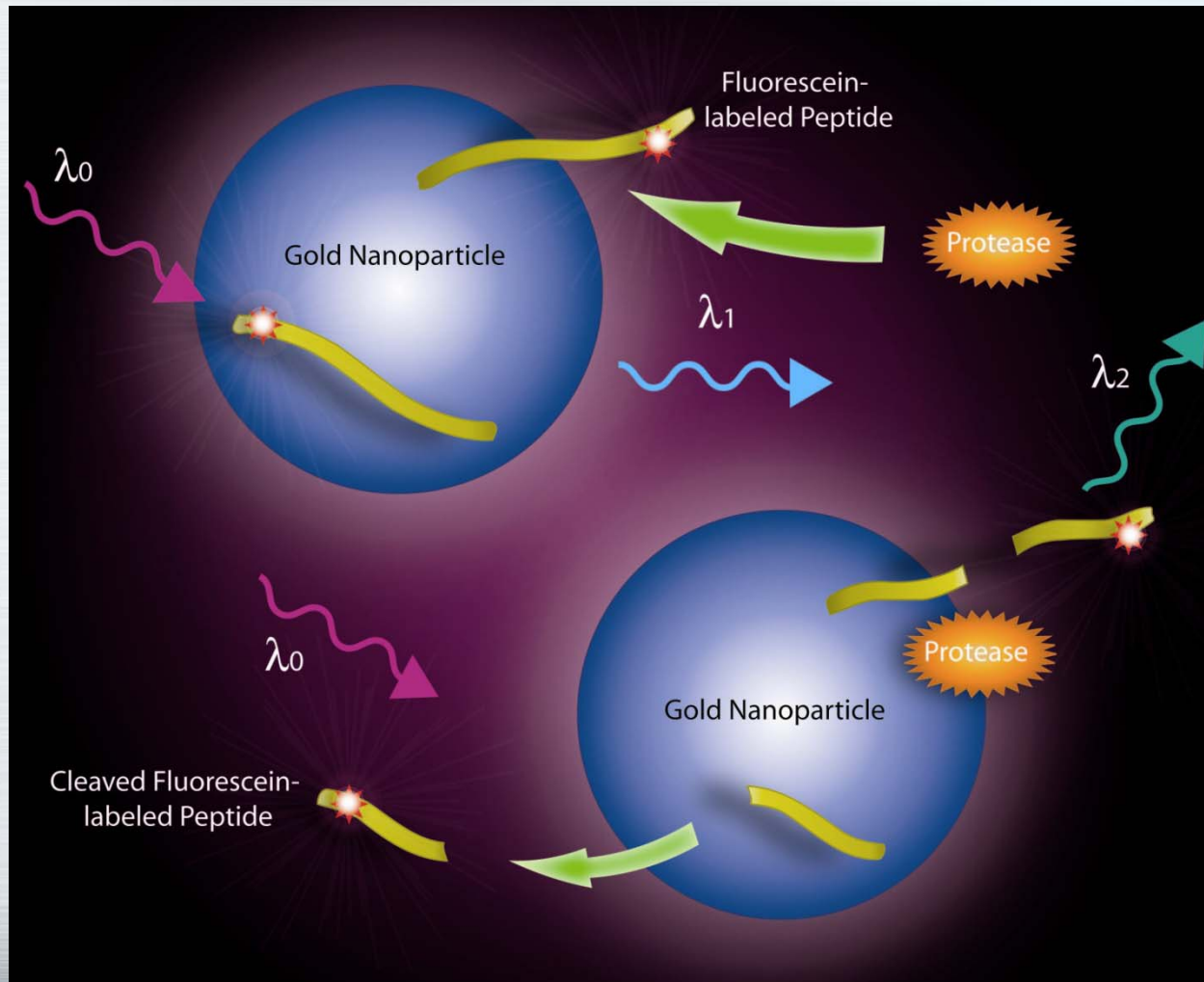


Surface Immobilization

- Simple covalent binding due to the strong interaction between gold and cysteine on the n-terminal of the peptide



Sensing Mechanism

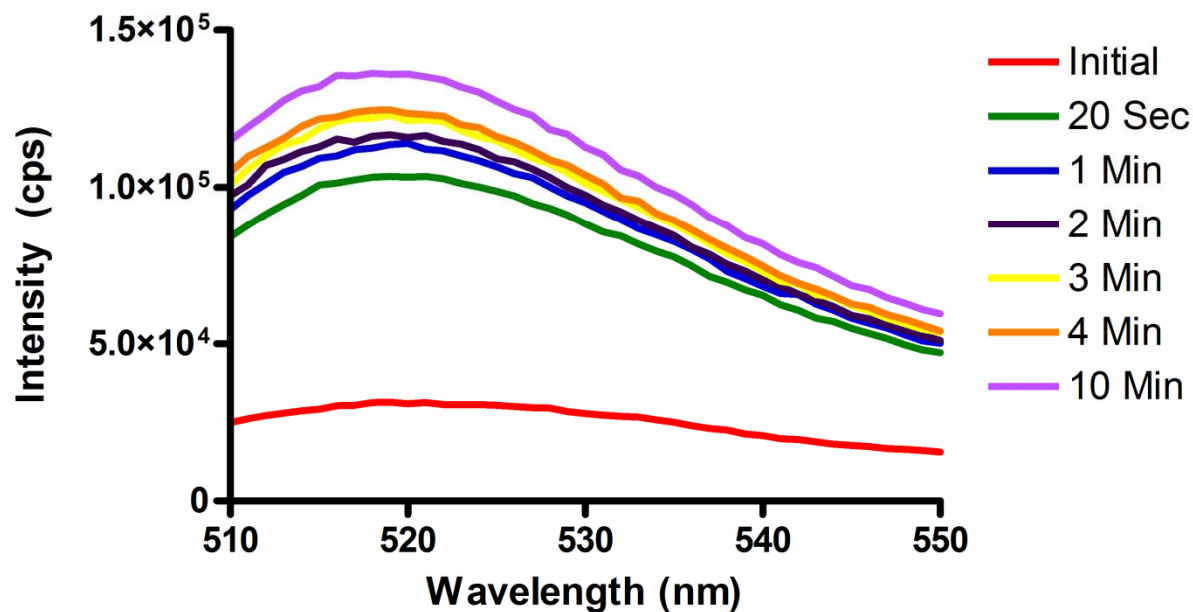


Results

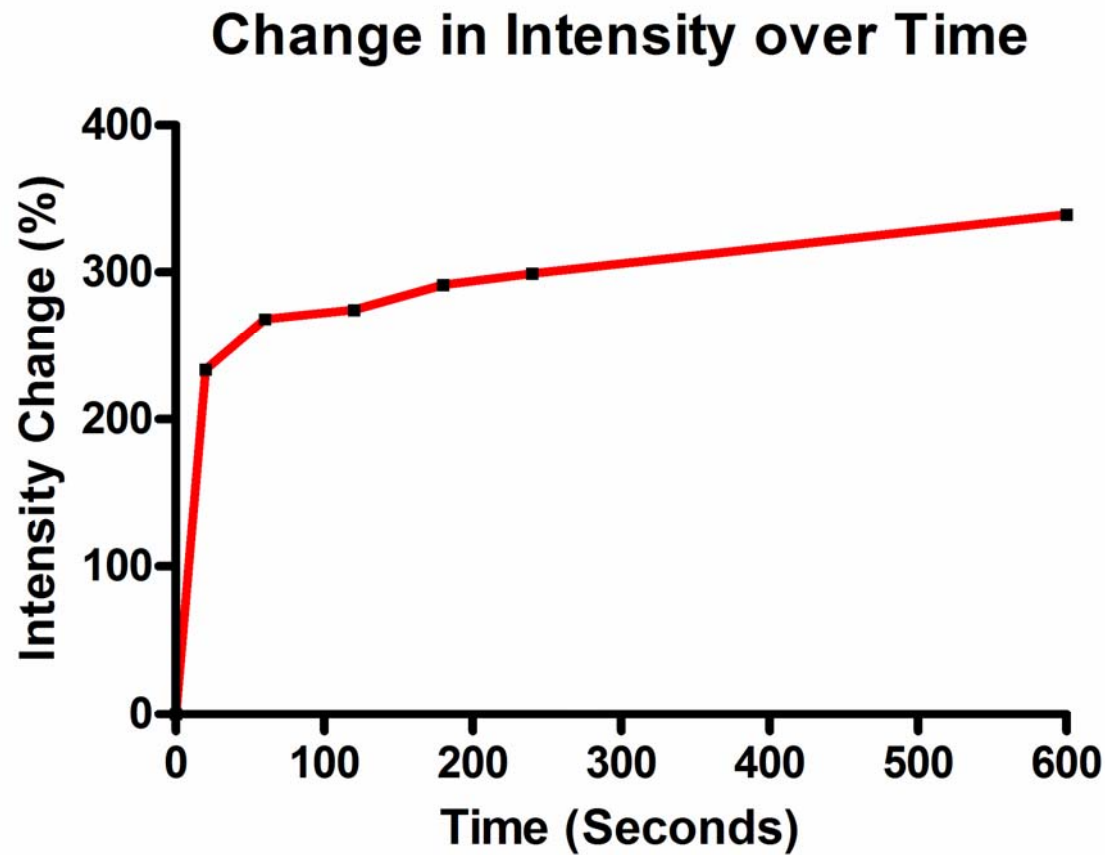


Fluorescence Recovery in Presence of Trypsin

Time Response - Trypsin Cleavage of Gold Nanoparticles/CF6 Peptide over Time

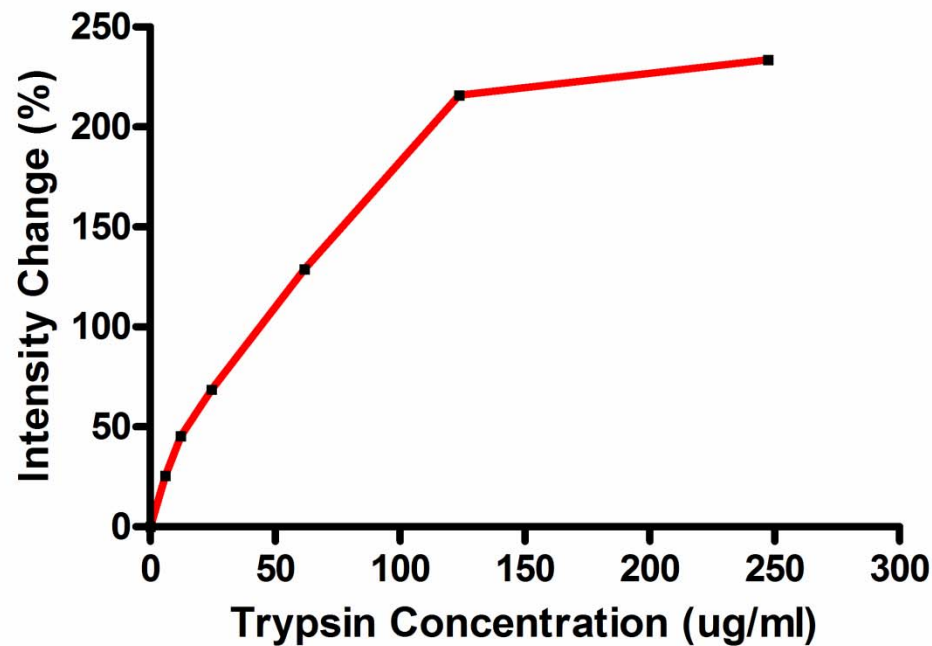


Percent Signal Change



Dosage Response

**Dosage Response - Percent Intensity Change
with Different Concentrations of Trypsin at 20 sec**



Conclusion

- It is possible to immobilize peptides onto metallic nanoparticles for a very fast and sensitive response to trypsin
- Dosage response indicated limit of detection in the range of $6.1 \mu\text{g/ml}$ with a near immediate response time



Future Work

- Continue to optimize conjugation protocol to decrease limit of detection
- Amend to detect additional analytes
 - Explosives (TNT, RDX, etc.)
 - Neurotoxins (Botulinum)
- Incorporating design into a lab-on-chip based device



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Questions?



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